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# Development of Grilled-type Shrimp Flavor by Maillard Reaction and Sensory Evaluation

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#### Abstract

A grilled-type shrimp flavor was developed through the Maillard reaction to reduce or mask fish odor or off-flavor in seafood. Model systems were created by using enzymatic hydrolysate of shrimp and adding precursors to increase flavor quality and stability. Amino acid precursors such as cysteine and methionine, sugar precursors such as glucose, xylose, ribose, and sucrose, and one particular compound of glucosamine were tried and their flavor qualities were tested by sensory evaluation. Also, the optimum reaction condition was investigated using the pH values of pH 5, 6, 7, and 8 with reaction times of 1 hr, 2 hr and 3 hr after the best precursors were determined. The best condition of the precursors for grilled-type shrimp flavor was the mixtures of methionine, threonine, xylose, and glucosamine. The optimum reaction condition was at pH 8.0 and 2 hr reaction time.

Key words: grilled-type shrimp flavor, reaction flavor, Alcalase, Maillard reaction, sensory evaluation

# **INTRODUCTION**

The consumption of shrimp and crab increases with economic growth. Shrimp and crab are known as high value seafood products, so their high quality is very important (1). While crustaceans such as crab and shrimp have a distinct taste and smell that are generally favorable for consumers (2), these flavors are reduced during storage and distribution. The fish smells and off-smells are increased as their freshness is decreased (3). A number of studies report on methods to inhibit these offsmells in the hopes of increasing the acceptance of seafood throughout processing, storage and distribution. Lee and Rhee (4) reported the effects of vinegar and ginger for reducing or masking fish smells, and Lee and Rhee (5), studied the effect of organic acids, such as acetic acid, for inhibiting the off-smell of salted clam pickle. Choi and Kang (6) developed the processing method for removing the fishy smell of sea eel. However, there are only a few studies on the masking effects of specific precursors in reaction flavor on fish smell. With this idea in mind, shrimp that have lots of protein, calcium and several vitamins were enzymatically hydrolyzed and the hydrolysate was used for reaction flavor with some precursors. The reaction flavor was expected to mask fishy odor and off-flavor, to improve natural shrimp flavors and to increase consumer acceptance of shrimp

# MATERIALS AND METHODS

#### Materials

The shrimp used for this study was chosen as spotted shrimp (*Trachysalambria curvirostris*), harvested near Korean seas. The shrimps were purchased at a relatively low price from a local market. The length and weight

flavor. Shrimp flavor types can be separated into boiled and grilled, depending on their preparation. The former has a strong burnt flavor and is usually used for ramen-based powder, seafood sauce, and surimi, as well as other items. The latter has a strong popcorn flavor and is mainly used for seasoning, snacks, and cookies. The differences in the volatile compounds of boiled-type and grilled-type shrimp flavors are known to be due to the different reaction pathways for each reaction process. Particularly, pyrazine compounds that are formed during the Maillard reaction are known to be important for burnt and popcorn flavor (7,8). The amount and kinds of pyrazines of the reaction flavor are reported to be different depending on precursors and reaction conditions (9). This study was performed to develop grilled-type shrimp flavor by determining the optimum precursors in the Maillard reaction, such as amino groups and sugar groups, and investigating the optimum reaction conditions such as pH and reaction time.

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of the shrimp samples were  $5 \sim 6$  cm and  $3 \sim 4$  g, respectively. The fresh shrimps were freeze-dried prior to the experiment.

# **Enzymatic hydrolysis**

The enzymatic hydrolysate of the shrimp samples was prepared by the modified method of Oh (10). Six grams of spotted shrimp were chopped and 40 mL of distilled water added. The autolytic enzyme of shrimp solution was deactivated by heating at 85°C for 15 min. The pH was adjusted to 5.0, 6.0, 7.0 and 8.0 with 2 M HCl or NaOH. Forty microliters of the commercial protease Alcalase 2.4LFG (Novo, Denmark) kept at 5°C was added and the sample solution was hydrolyzed for 30 min. Then, the hydrolyzed shrimp solution was again deactivated by heating it at 85°C for 15 min. The solution was centrifuged at 4,000 rpm for 40 min. The supernatant was used for reaction flavor experiments.

# Preparation of precursor mixtures

The mixing ratios of cysteine · HCl and methionine used for grilled-type reaction flavor are shown in Table 1 and their effects for shrimp reaction flavor were compared by sensory evaluation. For grilled-type shrimp flavor, threonine was also investigated as an amino precursor (Table 1). For reducing sugars, glucose, xylose, ribose and sucrose were investigated. The best precursors of amino acid and sugar were chosen by their highest sensory score and the closest grilled shrimp flavors. Another precursor of glucosamine that has both amino group and aldehyde group in a molecule was investigated for grilled-type shrimp flavor. For each step of sensory evaluation, the best precursors of amino acid and sugar that had the highest total sensory score and the closest grilled shrimp flavor were chosen. The reaction condition of model system for determining precursors was 100°C, a 1:1 ratio of amino acid and sugar. The reaction time and pH for model systems were 2 hr and pH 7.0, respectively. The reaction solution was distilled water or enzymatic hydrolysate.

## **Processing condition**

The processing conditions of the precursors determined for grilled-type shrimp flavor from model systems were investigated. Results are shown in Table 2. The reaction pH values were 5.0, 6.0, 7.0, and 8.0. The best pH was chosen using the results of the sensory evaluation. At the determined pH, the reaction times were 1 hr, 2 hr, and 3 hr.

## Sensory evaluation

Sensory evaluation was carried out to investigate the best reaction conditions to create a grilled-type shrimp flavor. The sensory panel was composed of 9 students from Pukyong National University. The sensory time was mainly carried out at about 4 pm in order to increase

Table 1. Experimental design on adding precursors for grilled-type shrimp flavor

T 1. ( )			Additio	on of cysteir	ne and methic	onine		
Ingredient (g)	Α	E		C	D		E	F
Cysteine·HCl	0.3	_	-	0.15	0.3		_	0.15
Methionine	_	0.	3	0.15	_	(	0.3	0.15
Water	50.0	50.	0	50.0	—		—	—
Shrimp hydrolysate	_	-	-	_	50.0	50	0.0	50.0
Glucose	0.3	0.	3	0.3	0.3	(	0.3	0.3
Total	50.6	50.	6	50.6	50.6	50	0.6	50.6
Ingredient (g)			Additio	n of methio	nine and thre	onine		
nigredient (g)	А	E	8	С	D		Е	F
Methionine	0.3	0.	15	_	0.3	(	0.15	_
Threonine	_	0.	15	0.3	—	(	0.15	0.3
Water	50.0	50.	0	50.0	_		_	_
Shrimp hydrolysate	_	_		_	50.0	50	0.0	50.0
Glucose	0.3	0.	3	0.3	0.3	(	0.3	0.3
Total	50.6	50.	6	50.6	50.6	50	0.6	50.6
Ingredient (g)			А	ddition of re	n of reducing sugar			
nigredient (g)	А	В	С	D	Е	F	G	Н
Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Glucose	0.3	_	_	_	0.3	-	_	_
Xylose	_	0.3	-	_	_	0.3	_	_
Ribose	_	_	0.3	_	_	-	0.3	_
Sucrose	-	_	—	0.3	_	_	—	0.3
Water	50.0	50.0	50.0	50.0	—	-	-	—
Shrimp hydrolysate	_	_			50.0	50.0	50.0	50.0
Total	50.6	50.6	50.6	50.6	50.6	50.6	50.6	50.6

In anadiant (a)		Addit	tion of glucosa	nine and sucros	e			
Ingredient (g)	1	2	3	4	5	6		
Methionine	0.15	0.1	_	0.15	0.1	_		
Threonine	0.15	0.1	—	0.15	0.1	_		
Sucrose	0.3	0.3	0.3	0.3	0.3	0.3		
Glucosamine	-	0.1	0.3	_	0.1	0.3		
Water	50.0	50.0	50.0	_	_	_		
Shrimp hydrolysate	—	_	_	50.0	50.0	50.0		
Total	50.6	50.6	50.6	50.6	50.6	50.6		
In and it and (a)	Addition of glucosamine and xylose							
Ingredient (g)	1	2	3	4	5	6		
Methionine	0.15	0.1	_	0.15	0.1	_		
Threonine	0.15	0.1	_	0.15	0.1	_		
Xylose	0.3	0.3	0.3	0.3	0.3	0.3		
Glucosamine	-	0.1	0.3	—	0.1	0.3		
Water	50.0	50.0	50.0	—	_	-		
Shrimp hydrolysate	_	_	_	50.0	50.0	50.0		
Total	50.6	50.6	50.6	50.6	50.6	50.6		

Table 2. Experimental design for the optimum reaction condition of grilled-type shrimp flavor

In anodiant (a)		Reaction pH				Reaction time (hr)		
Ingredient (g)	5.0	6.0	7.0	8.0	1	2	3	
Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
Threonine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
Xylose	0.3	0.3	0.3	0.3	0.3	0.3	0.3	
Glucosamine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
Shrimp hydrolysate	50.0	50.0	50.0	50.0	50.0	50.0	50.0	
Total	50.6	50.6	50.6	50.6	50.6	50.6	50.6	

sensory sensitivity. Each sample was placed in a transparent container (5.0 cm inside diameter  $\times$  7.0 cm height). Each panel directly smelled the sample and then tested it again with smelling blotter. Its evaluation was 9 grade method: 1 point is very weak, 5 point is normal, 9 point is very strong. The statistical analysis of these data was analyzed using completely randomized design (11). Their multiple comparisons were carried out with Duncan method at the significance level of  $\alpha$ =0.05 (12).

# **RESULTS AND DISCUSSION**

Sulfur-containing compounds are important for a particular seafood reaction flavor. For grilled-type shrimp flavor, cysteine, HCl and methionine of sulfur-containing amino acids were tried as an amino acid precursor (13). In order to increase the formation of pyrazine compounds of roast aroma, threonine of amino alcohol was considered as a precursor for grilled-type shrimp flavors (14). For sugar precursors, glucose, xylose, ribose, and sucrose were tested and glucosamine was also included. Glucosamine is the main component of chitin, which is mainly found in crustacean shells (15). Glucosamine may possibly contribute to the formation of reaction flavor from shrimp hydrolysate. Also, glucosamine is considered to be directly involved in the Maillard reaction as a precursor because it is aldose amine compound of Heynes, a compound which has both an amino group and aldehyde group in its molecular structure. The temporary reaction condition was 2 hr at 100°C.

## Determination of amino acid precursors

The quality of the reaction flavors upon the addition of cysteine · HCl and methionine into the model system and shrimp hydrolysate was tested by sensory evaluation. These results are shown in Table 3. The cysteine · HCl precursor showed the very strong sulfuric pungent smell of 7.6 points in the sensory analysis, and the reaction flavor of methionine precursor got a high score of 6.7 points in boiled potato smell. Cysteine · HCl, the sulfur-containing amino acid, is reported to produce mercapto compounds by Strecker degradation in Maillard reactions (16). These mercapto compounds have pungent aromas and are reported to be responsible in the formation of meat flavor (16,17). Methionine produces methional by Strecker degradation (16) and methional is known to produce boiled potato and vegetable flavors. (17).

Table 3. Sensory results of grilled-type shrimp flavor on adding amino acid precursors

Model systems of reaction flavor with the addition of cysteine HCl or methionine

Catagory	Model systems <sup>1)</sup>							
Category	1	2	3	4	5	6		
Total preference	$1.9^{d2}$	6.2 <sup>ab</sup>	3.1 <sup>cd</sup>	4.7 <sup>bc</sup>	6.7 <sup>a</sup>	5.8 <sup>b</sup>		
Boiled potato	$1.2^{b}$	$6.7^{a}$	$2.4^{b}$	2.3 <sup>b</sup>	3.0 <sup>b</sup>	$2.1^{b}$		
Boiled vegetable	$1.0^{b}$	3.7 <sup>a</sup>	$2.0^{ab}$	$1.9^{ab}$	$2.6^{a}$	$1.8^{b}$		
Sulfuric pungent	7.6 <sup>a</sup>	2.1 <sup>d</sup>	6.3 <sup>ab</sup>	$4.4^{bc}$	2.1 <sup>d</sup>	3.7 <sup>c</sup>		
Boiled shrimp	$1.1^{c}$	3.4 <sup>b</sup>	1.3 <sup>c</sup>	4.6 <sup>b</sup>	5.9 <sup>a</sup>	5.8 <sup>a</sup>		
Grilled shrimp	$1.2^{c}$	4.1 <sup>b</sup>	$1.2^{c}$	5.2 <sup>ab</sup>	7.2 <sup>a</sup>	5.9 <sup>a</sup>		
Balance of flavor	$2.0^{\circ}$	5.6 <sup>ab</sup>	$2.4^{\circ}$	4.9 <sup>b</sup>	6.8 <sup>a</sup>	5.8 <sup>a</sup>		

<sup>1)</sup>1: 0.3 g cysteine  $\pm$  0.3 g glucose  $\pm$  50 mL water, 2: 0.3 g methione  $\pm$  0.3 g glucose  $\pm$  50 mL water, 3: 0.15 g cysteine  $\pm$  0.15 g methionine  $\pm$  0.3 g glucose  $\pm$  50 mL water, 4: 0.3 g cysteine  $\pm$  0.3 g glucose  $\pm$  50 mL enzymatic hydrolysate of shrimp, 5: 0.3 g methionine  $\pm$  0.3 g glucose  $\pm$  50 mL enzymatic hydrolysate of shrimp, 6: 0.15 g cysteine  $\pm$  0.15 g methionine  $\pm$  0.3 g glucose  $\pm$  50 mL enzymatic hydrolysate of shrimp, 6: 0.15 g cysteine  $\pm$  0.15 g methionine  $\pm$  0.3 g glucose  $\pm$  50 mL enzymatic hydrolysate of shrimp, 6: 0.15 g cysteine  $\pm$  0.15 g methionine  $\pm$  0.3 g glucose  $\pm$  50 mL enzymatic hydrolysate of shrimp.

<sup>2)</sup>The different superscript letters within the same row for the average values mean significantly different at the p<0.05 level of significance as determined by Duncan's multiple range test.

The sensory scores for the quality of reaction flavors upon the addition of cysteine  $\cdot$  HCl and methionine into enzymatic hydrolysate of spotted shrimp were 4.4 in sulfuric pungent smell and 4.7 total preference. Sensory scores for reaction flavor of adding methionine were 3.0 and 6.7 in boiled potato smell and total preference, respectively. In the case of adding the 1:1 ratio of cysteine  $\cdot$  HCl and methionine, the sensory score of total preference was 5.8. Therefore, the reaction flavor of seafood type scored higher sensory points with the addition of methionine than those with the addition of cysteine  $\cdot$  HCl, which showed different results from the reaction flavor of meat type. Methionine is determined as an amino acid precursor in grilled-type shrimp flavors.

Flavor quality of reaction flavors for grilled-type shrimp flavor upon the addition of methionine and threonine into the model system and shrimp hydrolysate was evaluated and their sensory results are shown in Table 3. Initially, grilled-type shrimp flavor is thought to have "baked nutty aroma", such as what is given by pyrazine compounds, more so than those of boiled-type shrimp flavor. In the total preference of sensory results, the reaction flavors for shrimp hydrolysate systems of amino acid precursors had 4.4, 5.9 and 5.1 for methionine, 1:1 of methionine and threonine, and threonine, respectively, showed a big difference from the model systems using distilled water. The boiled aroma of methional from methionine, and the baked aroma of pyrazine from threonine are thought to make grilled-type shrimp flavor bet-

<sup>(2)</sup> Model systems of reaction flavor with the addition of threonine

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Catagory	Model systems <sup>1)</sup>							
Category	1	2	3	4	5	6		
Total preference	6.3 <sup>a2)</sup>	$4.8^{ab}$	$3.0^{c}$	4.4 <sup>b</sup>	5.9 <sup>a</sup>	5.1 <sup>ab</sup>		
Boiled potato	6.4 <sup>a</sup>	$5.0^{a}$	$1.6^{b}$	$2.4^{b}$	2.3 <sup>b</sup>	2.1 <sup>b</sup>		
Boiled vegetable	$2.5^{a}$	$2.4^{a}$	$1.6^{a}$	$1.9^{a}$	$1.9^{a}$	$2.1^{a}$		
Sulfuric pungent	1.4 <sup>b</sup>	1.1 <sup>b</sup>	1.3 <sup>b</sup>	$2.4^{a}$	$2.0^{ab}$	$2.4^{a}$		
Boiled shrimp	1.5 <sup>b</sup>	$1.0^{b}$	$1.0^{b}$	5.1 <sup>a</sup>	5.4 <sup>a</sup>	$5.0^{a}$		
Grilled shrimp	2.6 <sup>b</sup>	$1.5^{bc}$	$1.0^{c}_{1.0}$	$6.6^{a}$	6.9 <sup>a</sup>	6.6 <sup>a</sup>		
Fishy smell	1.6 <sup>b</sup>	1.9 <sup>b</sup>	1.5 <sup>b</sup>	$2.9^{ab}$	$2.9^{ab}$	4.3 <sup>a</sup>		
Balance of flavor	5.6 <sup>a</sup>	4.6 <sup>a</sup>	2.3 <sup>b</sup>	5.1 <sup>a</sup>	6.1 <sup>a</sup>	5.1 <sup>a</sup>		

<sup>1)</sup>1: 0.3 g methionine +0.3 g glucose + 50 mL water, 2: 0.15 g methionine + 0.15 g threonine + 50 mL water, 3: 0.3 g threonine + 50 mL water, 4: 0.3 g methionine + 0.3 g glucose + 50 mL enzymatic hydrolysate of shrimp, 5: 0.15 g methionine + 0.15 g threonine + 50 mL enzymatic hydrolysate of shrimp, 6: 0.3 g threonine + 50 mL enzymatic hydrolysate of shrimp.

<sup>2)</sup>The different superscript letters within the same row for the average values mean significantly different at the p<0.05 level of significance as determined by Duncan's multiple range test.

ter, and the mixed precursor of the 1:1 ratio of methionine and threonine did prove to have the highest score for the total preference. Therefore, methionine and threonine are determined to be the most desirable amino acid precursors for grilled-type shrimp flavors.

#### Determination of sugar precursors

Flavor quality for grilled-type shrimp flavors upon the addition of glucose of hexose, xylose and ribose of pentose, sucrose of disaccharide into the model system and shrimp hydrolysate was evaluated and their results are shown in Table 4. In the sensory results of the model systems, the reaction flavors of adding glucose, xylose, ribose and sucrose with the mixture of methionine and threonine had scores of 3.4, 3.9, 3.9 and 4.6 points for total preference, respectively. The quality of reaction flavors upon the addition of sugars with the mixture of methionine and threonine into the enzymatic hydrolysate of spotted shrimp resulted in total preference sensory scores of 6.0, 7.6, 6.7 and 8.0 points for glucose, xylose, ribose and sucrose, respectively. As shown in Table 5, the sensory scores of the total preference for reaction flavor of glucosamine and xylose were higher than those of either glucosamine or sucrose, so xylose was determined to be the most desirable sugar precursor for grilled-type shrimp flavor.

### Application of glucosamine

Sensory results upon the addition all the precursors of methionine, threonine, xylose and glucosamine are

Catagory				Model s	ystems <sup>1)</sup>			
Category	1	2	3	4	5	6	7	8
Total preference	$3.4^{d2)}$	3.9 <sup>d</sup>	3.9 <sup>d</sup>	4.6 <sup>cd</sup>	6.0 <sup>bc</sup>	7.6 <sup>ab</sup>	6.7 <sup>ab</sup>	$8.0^{a}$
Boiled potato	3.7 <sup>b</sup>	3.9 <sup>b</sup>	4.7 <sup>a</sup>	6.3 <sup>a</sup>	3.0 <sup>b</sup>	3.7 <sup>b</sup>	3.9 <sup>b</sup>	3.7 <sup>b</sup>
Boiled vegetable	3.6 <sup>a</sup>	4.3 <sup>a</sup>	4.1 <sup>a</sup>	5.0 <sup>a</sup>	3.1 <sup>a</sup>	3.7 <sup>a</sup>	3.6 <sup>a</sup>	3.3 <sup>a</sup>
Sulfuric pungent	$2.9^{a}$	3.1 <sup>a</sup>	$2.7^{\mathrm{a}}$	3.3 <sup>a</sup>	2.3 <sup>a</sup>	3.1 <sup>a</sup>	$2.9^{a}$	2.4 <sup>a</sup>
Boiled shrimp	$2.0^{\circ}$	$1.9^{\circ}$	$2.3^{\circ}$	$2.7^{\circ}$	5.4 <sup>b</sup>	5.9 <sup>b</sup>	5.7 <sup>b</sup>	7.3 <sup>a</sup>
Grilled shrimp	$1.7^{c}$	$1.7^{c}$	$2.3^{\circ}$	$2.6^{\circ}$	5.9 <sup>b</sup>	7.4 <sup>a</sup>	7.1 <sup>ab</sup>	7.7 <sup>a</sup>
Fishy smell	$1.3^{c}$	$1.1^{c}_{1.1}$	$1.1^{c}_{1.1}$	$1.6^{c}_{1.6}$	3.4 <sup>a</sup>	$2.0^{abc}$	$2.9^{ab}$	$2.1^{abc}$
Balance of flavor	3.9 <sup>b</sup>	4.4 <sup>b</sup>	4.3 <sup>b</sup>	4.6 <sup>b</sup>	6.3 <sup>a</sup>	$7.6^{a}$	6.9 <sup>a</sup>	$7.6^{a}$

Table 4. Sensory results of grilled-type shrimp flavor with addition of reducing sugar precursors

<sup>1)</sup>1: 0.15 g methionine +0.15 g threonine +0.3 g glucose +50 mL water, 2: 0.15 g methionine +0.15 g threonine +0.3 g xylose +50 mL water, 3: 0.15 g methionine +0.15 g threonine +0.3 g ribose +50 mL water, 4: 0.15 g methionine +0.15 g threonine +0.3 g sucrose +50 mL water, 5: 0.15 g methionine +0.15 g threonine +0.3 g glucose +50 mL enzymatic hydrolysate of shrimp, 6: 0.15 g methionine +0.15 g threonine +0.3 g xylose +50 mL enzymatic hydrolysate of shrimp, 7: 0.15 g methionine +0.3 g sucrose +50 mL enzymatic hydrolysate of shrimp, 8: 0.15 g methionine +0.15 g threonine +0.3 g sucrose +50 mL enzymatic hydrolysate of shrimp, 8: 0.15 g methionine +0.15 g threonine +0.3 g sucrose +50 mL enzymatic hydrolysate of shrimp, 8: 0.15 g methionine +0.15 g threonine +0.3 g sucrose +50 mL enzymatic hydrolysate of shrimp, 8: 0.15 g methionine +0.15 g threonine +0.3 g sucrose +50 mL enzymatic hydrolysate of shrimp, 8: 0.15 g methionine +0.15 g threonine +0.3 g sucrose +50 mL enzymatic hydrolysate of shrimp.

<sup>2)</sup>The different superscript letters within the same row for the average values mean significantly different at the p<0.05 level of significance as determined by Duncan's multiple range test.

Table 5. Sensory results of grilled-type shrimp flavor with the addition of glucosamine precursor

 $\ensuremath{\mathbbm D}$  Model systems of reaction flavor on adding glucosamine and sucrose

Catagory	Model systems <sup>1)</sup>							
Category -	1	2	3	4	5	6		
Total preference	3.8 <sup>ab2)</sup>	$1.4^{c}$	$2.8^{bc}$	5.2 <sup>a</sup>	5.2 <sup>a</sup>	5.2 <sup>a</sup>		
Boiled potato	5.1 <sup>a</sup>	1.3 <sup>b</sup>	$4.6^{a}$	2.1 <sup>b</sup>	$1.9^{b}_{1}$	1.7 <sup>b</sup>		
Boiled vegetable	$3.6^{a}$	1.5 <sup>b</sup>	$3.5^{a}_{}$	$2.0^{b}$	1.7 <sup>b</sup>	1.7 <sup>b</sup>		
Sulfuric pungent	$2.1^{ab}$	1.1 <sup>b</sup>	$1.9^{ab}$	$2.6^{a}$	$2.3^{ab}$	$2.6^{a}$		
Boiled shrimp	$2.0^{b}_{1}$	1.1 <sup>b</sup>	$2.1^{b}_{1}$	5.7 <sup>a</sup>	4.3 <sup>a</sup>	$5.3^{a}$		
Grilled shrimp	2.3 <sup>b</sup>	1.1 <sup>b</sup>	2.3 <sup>b</sup>	6.1 <sup>a</sup>	5.4 <sup>a</sup>	$6.6^{a}$		
Fishy smell	$2.4^{a}$	$1.9^{a}$	$2.4^{a}$	3.4 <sup>a</sup>	$2.8^{a}$	$3.7^{a}$		
Balance of flavor	4.5 <sup>ab</sup>	1.8 <sup>c</sup>	$3.5^{bc}$	5.3 <sup>ab</sup>	$4.9^{ab}$	5.4 <sup>a</sup>		

<sup>1)</sup>1: 0.15 g methionine + 0.15 g threonine + 0.3 g sucrose + 50 mL water, 2: 0.3 g glucosamine + 0.3 g sucrose + 50 mL water, 3: 0.1 g methionine + 0.1 g threonine + 0.3 g sucrose + 0.1 g glucosamine + 50 mL water, 4: 0.15 g methionine + 0.15 g threonine + 0.3 g sucrose + 50 mL enzymatic hydrolysate of shrimp, 5: 0.3 g glucosamine + 0.3 g sucrose + 0.1 g glucosamine + 50 mL enzymatic hydrolysate of shrimp, 6: 0.1 g methionine + 0.1 g threonine + 0.1 g glucosamine + 0.3 g sucrose + 50 mL enzymatic hydrolysate of shrimp, 6: 0.1 g methionine + 0.1 g threonine + 0.1 g glucosamine + 0.3 g sucrose + 50 mL enzymatic hydrolysate of shrimp, 6: 0.1 g methionine + 0.1 g threonine + 0.1 g glucosamine + 0.3 g sucrose + 50 mL enzymatic hydrolysate of shrimp.

<sup>2)</sup>The different superscript letters within the same row for the average values mean significantly different at the p<0.05 level of significance as determined by Duncan's multiple range test.

shown in Table 5. In the sensory results of reaction flavors from shrimp hydrolysate, total preference had its highest score of 7.1 and the grilled shrimp smell was the strongest at 6.3 when methionine, threonine, glucosamine and xylose were added. Therefore, precursors for grilled-type shrimp flavors were determined to be methionine, threonine, xylose and glucosamine.

### Determination of optimum processing conditions

The quality of reaction flavor primarily depends on the precursors and reaction conditions used. Important parameters such as reaction condition, reaction pH and

② Model systems of reaction flavor with the addition of glucosamine and xylose

Catagory	Model systems <sup>1)</sup>							
Category	1	2	3	4	5	6		
Total preference	$3.9^{c2}$	3.3°	$4.4^{bc}$	6.1 <sup>ab</sup>	$6.3^{ab}$	7.1 <sup>ª</sup>		
Boiled potato	5.1 <sup>a</sup>	2.3 <sup>b</sup>	$4.2^{ab}$	3.6 <sup>ab</sup>	$2.9^{b}$	2.9 <sup>b</sup>		
Boiled vegetable	$4.0^{a}$	$2.4^{a}$	3.9 <sup>a</sup>	$2.9^{a}$	$2.3^{a}$	$2.1^{a}$		
Sulfuric pungent	$2.4^{a}_{.}$	$1.3^{a}_{}$	$3.0^{a}$	$2.3^{a}$	$2.1^{a}$	$2.4^{\mathrm{a}}$		
Boiled shrimp	$2.9^{b}$	$2.6^{b}$	2.7 <sup>b</sup>	5.7 <sup>a</sup>	5.3 <sup>a</sup>	5.9 <sup>a</sup>		
Grilled shrimp	$2.8^{\circ}$	$2.8^{\circ}$	$3.2^{bc}$	$5.4^{ab}$	6.1 <sup>a</sup>	6.3 <sup>a</sup>		
Fishy smell	$2.7^{a}_{}$	$2.1^{a}$	$2.6^{a}$	$2.6^{a}$	$2.6^{a}$	$2.4^{a}$		
Balance of flavor	4.1 <sup>bc</sup>	3.4 <sup>c</sup>	3.8 <sup>c</sup>	6.0 <sup>a</sup>	5.7 <sup>ab</sup>	6.3 <sup>a</sup>		

<sup>1)</sup>1: 0.15 g methionine + 0.15 g threonine + 0.3 g xylose + 50 mL water, 2: 0.3 g glucosamine + 0.3 g xylose + 50 mL water, 3: 0.1 g methionine + 0.1 g threonine + 0.3 g xylose + 0.1 g glucosamine + 50 mL water, 4: 0.15 g methionine + 0.15 g threonine + 0.3 g xylose + 50 mL enzymatic hydrolysate of shrimp, 5: 0.3 g glucosamine + 0.3 g xylose + 0.1 g glucosamine + 50 mL enzymatic hydrolysate of shrimp, 6: 0.1 g methionine + 0.1 g threonine + 0.1 g glucosamine + 0.3 g xylose + 50 mL enzymatic hydrolysate of shrimp, 6: 0.1 g methionine + 0.1 g threonine + 0.1 g glucosamine + 0.3 g xylose + 50 mL enzymatic hydrolysate of shrimp.

<sup>2)</sup>The different superscript letters within the same row for the average values mean significantly different at the p<0.05 level of significance as determined by Duncan's multiple range test.

time must be considered. Mottram and Whitfield (9) reported that the pH of meat, like with the Maillard system, is an important factor in the formation of sulfur-containing compounds and pyrazine compounds. In order to determine the optimum pH for boiled-type and grilled-type shrimp flavor, pH values of 5, 6, 7, and 8 at the reaction conditions of 100°C and 2 hr were each investigated. At the determined pH, the optimum reaction time was chosen by sensory evaluation on reaction flavors in the reaction time of 1, 2 and 3 hours.

Table 6. Sensory results of grilled-type shrimp flavor on reaction  $\ensuremath{\text{pH}}$ 

Catagory	Reaction pH						
Category	pH 5	pH 6	pH 7	pH 8			
Total preference	$2.4^{b1}$	3.2 <sup>b</sup>	6.7 <sup>a</sup>	7.6 <sup>a</sup>			
Boiled potato	1.9 <sup>a</sup>	$1.7^{a}$	$2.1^{a}$	$1.9^{a}$			
Boiled vegetable	1.3 <sup>a</sup>	1.3 <sup>a</sup>	$1.4^{a}$	$1.6^{a}$			
Sulfuric pungent	$2.7^{a}$	$2.2^{a}$	$1.4^{a}$	$1.4^{a}$			
Boiled shrimp	$2.4^{b}$	3.7 <sup>b</sup>	5.9 <sup>a</sup>	$5.8^{a}$			
Grilled shrimp	$2.6^{\circ}$	4.7 <sup>b</sup>	$6.9^{a}$	$7.6^{a}$			
Fishy smell	$2.9^{a}$	$2.8^{a}$	$2.1^{a}$	$2.0^{a}$			
Chemical flavor	7.1 <sup>a</sup>	$6.0^{a}$	$2.6^{b}$	2.2 <sup>b</sup>			
Soy sauce flavor	$5.8^{a}$	$5.0^{ab}$	$5.8^{a}$	$2.9^{b}$			
Balance of flavor	2.3 <sup>b</sup>	2.9 <sup>b</sup>	6.9 <sup>a</sup>	7.2 <sup>a</sup>			

<sup>1)</sup>The different superscript letters within the same row for the average values mean significantly different at the p < 0.05 level of significance as determined by Duncan's multiple range test.

### Determination of reaction pH

The quality of reaction flavors that were made at several reaction pH values was evaluated and the sensory results are shown in Table 6. In grilled-type shrimp flavors, the sensory scores for total preference were 2.4, 3.2, 6.7, and 7.6 at reaction pH 5, 6, 7, and 8, respectively, showing the big difference between acidic pH values (pH 5 and 6) and neutral or alkali pH values (pH 7 and 8). The chemical smell was increased as the reaction pH was decreased. This big difference is possibly due to the different intermediates formed during pH changes, so reaction pH is important for Maillard reaction and flavor formation. Mottram and Whitfield (9) reported that the types of volatile compounds formed depend on the reaction pH in meat like in the Maillard system, which showed that the reaction system in neutral pH produced more sulfur-containing compounds and pyrazine compounds than in acidic pH. Baltes (14) reported that basic ammonia increased the formation of N-heterocyclic compounds such as pyrazines and pyridines. The reaction flavors of model systems at pH 7.0 and pH 8.0 scored high for total preference. The model system at pH 8.0 showed a better quality of less off-flavor in soy sauce flavor. Therefore, the optimum reaction pH for grilled-type shrimp flavors was determined to be 8.0.

## Determination of reaction time

The quality of reaction flavors that were made at several reaction times was evaluated and the sensory results are shown in Table 7. In grilled-type shrimp flavors, the sensory scores in total preference were 7.1, 8.3, and 5.6 at reaction times of 1, 2 and 3 hr, respectively. Also, the scores on chemical flavor of reaction models for 1, 2, and 3 hr reaction times were 2.4, 1.5, and 3.9, respectively. Therefore, a two hour reaction time was

 
 Table 7. Sensory results of grilled-type shrimp flavor on reaction time

Catagory	Reaction time (hr)						
Category	1 hr	2 hr	3 hr				
Total preference	$7.1^{a1)}$	8.3 <sup>a</sup>	5.6 <sup>b</sup>				
Boiled potato	2.3 <sup>a</sup>	1.5 <sup>a</sup>	1.4 <sup>a</sup>				
Boiled vegetable	$1.8^{\mathrm{a}}$	1.3 <sup>a</sup>	1.3 <sup>a</sup>				
Sulfuric pungent	$2.4^{a}$	$3.0^{a}$	$2.4^{a}$				
Grilled shrimp	$6.0^{ab}$	$7.0^{a}$	4.8 <sup>b</sup>				
Fishy smell	$1.8^{a}$	$1.4^{a}$	1.5 <sup>a</sup>				
Chemical flavor	$2.4^{ab}$	1.5 <sup>b</sup>	3.9 <sup>a</sup>				
Soy sauce flavor	$3.8^{\mathrm{a}}$	4.5 <sup>a</sup>	3.8 <sup>a</sup>				
Balance of flavor	6.8 <sup>ab</sup>	$7.9^{a}$	5.5 <sup>b</sup>				

<sup>1)</sup>The different superscript letters within the same row for the average values mean significantly different at the p < 0.05 level of significance as determined by Duncan's multiple range test.

determined to be optimum.

# CONCLUSION

Grilled-type shrimp flavor was prepared by adding the precursors of methionine, threonine, glucosamine, and xylose into shrimp hydrolysate and then allowing them to react at pH 8.0 and 100°C for a 2 hour reaction time, showing different precursors from those of boiled-type shrimp flavor. Further study on the differences of the volatile compounds from both boiled-type and grilled-type shrimp flavors is needed to investigate the grilled-type shrimp flavor, which can be used for shrimp snacks, natural seasoning, cookies and bread, and others. In addition, future studies on glucosamine for grilled-type shrimp flavor is also important in order to evaluate its reaction mechanism as a precursor.

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